

In the Claims

Claim 1 (currently amended). A method of concentrating, detecting, and extracting particles from a whole blood sample, the method comprising:

exposing the blood sample to an enzyme-detergent combination comprising plasminogen and streptokinase, wherein said plasminogen and streptokinase is maintained in a frozen state in coincident relation until exposure of the blood sample to the enzyme-detergent combination and wherein said streptokinase reacts with plasminogen upon thawing whereby plasmin is formed; and analyzing the exposed blood sample for the presence of particles.

Claim 2-3 (canceled).

Claim 4 (currently amended). The method of claim 1~~claim 3~~ wherein the plasminogen is suspended in an aqueous salt solution prior to freezing.

Claim 5 (original). The method of claim 4 wherein the aqueous salt solution comprises NaCl.

Claim 6 (previously presented). The method of claim 4 wherein the aqueous salt solution comprises Na_3PO_4 .

Claim 7 (previously presented). The method of claim 1 wherein the particles are selected from the group consisting of prions, toxins, metabolic markers, cancerous matter, disease state markers, bacteria, virus, and fungi.

Claim 8 (previously presented). The method of claim 1 wherein the particles are DNA molecules and further comprising the step of replicating the particles through PCR.

Claim 9 (currently amended). The method of claim 1 further comprising the step of exposing the whole blood sample to a DNase.

Claim 10 (currently amended). The method of claim 1 further comprising the step of exposing the whole blood sample to an endonuclease.

Claim 11 (currently amended). The method of claim 1~~claim 2~~ wherein the plasminogen and streptokinase are in a separate and dried state.

Claim 12 (original). The method of claim 11 wherein the plasminogen and streptokinase are mixed and distributed in disposable test containers.

Claim 13 (previously presented). The method of claim 11 wherein the plasminogen is combined with one or more enzymes selected from the group consisting of Phospholipase A₂, DNase, Endonuclease, and Lipase.

Claim 14 (currently amended). The method of claim 13 wherein the enzyme-detergent combination is suspended then dried in pellets of trehalose storage buffer and packaged as a dry reagent.

Claim 15 (currently amended). The method of claim 11 wherein the streptokinase is suspended then dried in pellets of trehalose storage buffer and packaged into tubes as a dry reagent.

Claim 16 (currently amended). The method of claim 11 further comprising:
resuspending the plasminogen and streptokinase in a buffer solution;
adding the buffer solution containing plasminogen and streptokinase to the ~~volume of~~ whole
blood sample; and
incubating the whole blood samples~~sample~~ at room temperature.

Claim 17 (currently amended). The method of claim 16, wherein the enzymes of the enzyme-detergent combination are in a dried state and comprise any combination of 1,500-4,500 KU Phospholipase A₂, 5,000-10,000 U Streptokinase, 2-10 U Plasminogen, 200-3,650 U DNase, 200-4,000 U Endonuclease, and 10,000-100,000 U Lipase.

Claim 18 (currently amended). The method of claim 16 further comprising:
centrifuging the incubated whole blood sample~~buffer solution~~ to form a supernatant and a pellet;
decanting the supernatant from the centrifuged whole blood sample~~buffer solution~~; and
washing the pellet.

Claim 19 (currently amended). The method of claim 18 wherein the whole blood sample~~buffer solution~~ is centrifuged for approximately 20 minutes at 5,000-5,500 x g at a temperature of 10-20°C.

Claim 20 (currently amended). The method of claim 18 wherein the pellet is washed with an Ecotine-HEPES solution, or a Sucrose-HEPES solution, or an Ecotine-HEPES solution and a Sucrose-HEPES solution.

Claim 21-22 (canceled).

Claim 23 (currently amended). The method of claim 16 further comprising:
centrifuging the ~~buffer solution~~incubated whole blood sample to form a supernatant and a pellet;
decanting the supernatant from the centrifuged ~~buffer solution~~whole blood sample;
digesting the ~~sample~~pellet; and
applying the ~~sample~~digested pellet to a nucleic acid extraction method.

Claim 24 (currently amended). The method of claim 23 wherein digesting the sample pellet further comprises lysis and DNase inactivation.

Claim 25 (currently amended). The method of claim 23 wherein digesting the sample pellet further comprises lysis and Endonuclease inactivation.

Claim 26 (currently amended). The method of claim 23 wherein digesting the sample pellet further comprises utilizing proteinase K, sodium dodecyl sulfate, aurintricarboxylic acid, and sodium citrate buffer, incubated at room temperature.

Claim 27-32 (canceled).

Claim 33 (currently amended). The method of claim 16 wherein the buffer solution comprises Potassium Phosphate, Magnesium Chloride, Sodium Chloride, and Aurintricarboxylic Acid.

Claim 34 (currently amended). The method of claim 33 wherein the buffer solution further comprises octylphenol ethoxylate.

Claim 35 (currently amended). The method of claim 33 wherein the enzyme-detergent combination comprises a trehalose storage buffer.

Claim 36 (currently amended). The method of claim 35 wherein methyl 6-O-(N-heptylcarbamoyl)- α -D-glucopyranoside and Saponin are provided in the trehalose storage buffer.

Claim 37 (currently amended). The method of claim 36 wherein the concentration of methyl 6-O-(N-heptylcarbamoyl)- α -D-glucopyranoside in the trehalose storage buffer is 20-35mM.

Claim 38 (currently amended). The method of claim 36 wherein the concentration of Saponin in the trehalose storage buffer is 0.05-0.1%.

Claim 39 (previously presented). The method of claim 35 wherein the trehalose storage buffer comprises Potassium Phosphate, octylphenol ethoxylate, Dithiothreitol, and Trehalose.

Claim 40 (original). The method of claim 39 wherein the trehalose storage buffer comprises 10 mM Potassium Phosphate.

Claim 41 (previously presented). The method of claim 39 wherein the trehalose storage buffer comprises 0.01-0.04% octylphenol ethoxylate.

Claim 42 (original). The method of claim 39 wherein the trehalose storage buffer comprises 1-5 mM Dithiothreitol.

Claim 43 (original). The method of claim 39 wherein the trehalose storage buffer comprises 0.3-0.5 M Trehalose.

Claim 44 (currently amended). The method of claim 1 wherein prior to exposing the whole blood sample to said an enzyme-detergent combination the blood is contacted with an anticoagulant.

Claim 45 (currently amended). The method of claim 1 wherein the whole blood exposed to said the enzyme-detergent combination is unclotted whole blood.

Claim 46 (previously presented). The method of claim 1 wherein the whole blood sample is at least 6.0 ml.

Claim 47 (previously presented). The method of claim 1 wherein the enzyme-detergent combination comprises a Phospholipase A₂ and a DNase.

Claim 48 (currently amended). The method of claim 47 further comprising exposing the whole blood sample to methyl 6-O-(N-heptylcarbamoyl)- α -D-glucopyranoside and Saponin.

Claim 49 (currently amended). The method of claim 1 further comprising exposing the whole blood sample to a DNase and aurintricarboxylic acid.

Claim 50 (previously presented). The method of claim 33 wherein the method is conducted at pH 7.8 to 8.0.

Claim 51 (new). A method of facilitating down stream extraction and detection of proteins or nucleic acids derived from a microorganism present in a blood sample, said method comprising contacting a blood sample with an enzyme cocktail comprising Phospholipase A₂, DNase, and aurintricarboxylic acid (ATA).

Claim 52 (new). The method according to claim 51, wherein prior to contacting the blood sample with said enzyme cocktail the blood is contacted with an anticoagulant.

Claim 53 (new). The method according to claim 51, wherein the blood contacted with said enzyme cocktail is unclotted blood.

Claim 54 (new). The method according to claim 51, wherein the blood sample is at least 6.0 ml.

Claim 55 (new). The method according to claim 51, wherein methyl 6-O-(N-heptylcarbamoyl)- α -D-glucopyranoside and Saponin are added to said enzyme cocktail.

Claim 56 (new). The method according to claim 51, wherein an endonuclease is substituted for DNase.

Claim 57 (new). The method according to claim 51, wherein an enzyme that digests nuclear membranes of white blood cells is substituted for Phospholipase A₂.

Claim 58 (new). The method according to claim 51, wherein said enzyme cocktail further comprises streptokinase and plasminogen.

Claim 59 (new). The method according to claim 51, wherein said microorganism is a virus.

Claim 60 (new). The method according to claim 51, wherein said microorganism is a fungus.

Claim 61 (new). The method according to claim 51, wherein said microorganism is a bacterium.

Claim 62 (new). The method according to claim 51, wherein bioactive peptides functionalized on a surface are used to capture bacteria or fungi present in blood that is contacted with said enzyme cocktail.

Claim 63 (new). The method according to claim 51, wherein aurintricarboxylic acid is provided at 25 – 80mM final concentration in order to promote extensive polymerization of the aurintricarboxylic acid which then allows DNase activity to not be inhibited when DNase is added, whereas said aurintricarboxylic acid provides protection of DNA inside blood borne microorganisms from the added DNase.

Claim 64 (new). The method according to claim 11, wherein the enzyme-detergent combination further comprises an enzyme that can break down a nuclear membrane.

Claim 65 (new). The method according to claim 1, wherein the method is conducted at pH 7.8 to 8.0.